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APPLICATION NUMBER: *60/479,852*
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
to follow		

☐ Additional inventors are being named on the ____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

OIL SEED MEAL PREPARATION

Direct all correspondence to CORRESPONDENCE ADDRESS

☒ Customer Number 24223 →

OR Type Customer Number here

Bar Code Label here 24223

PATENT TRADEMARK OFFICE

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	Number of Pages 11	☐ CD(s), Number	
☒ Drawing(s)	Number of Sheets 4	☒ Other (specify)	Postcard
☒ Application Data Sheet See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

		FILING FEE AMOUNT ($)
☒	Applicant claims small entity status. See 37 CFR 1.27.	$80.00
☐	A check or money order is enclosed to cover the filing fees	
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☐	Payment by credit card. Form PTO-2038 is attached.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

☒ No

☐ Yes, the name of the U S Government agency and the Government contract number are: _____

Respectfully submitted,

Date 20-June-03

SIGNATURE

REGISTRATION NO (if appropriate) 24,973

TYPED or PRINTED NAME Michael I. Stewart

Docket Number 7865-73 MIS

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

PTOSMALL/REV05

INITIAL INFORMATION DATA SHEET

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Application Information

Title Line One:	OIL SEED MEAL PREPARATION
Total Drawing Sheets:	Four (4)
Formal Drawings?:	YES
Application Type:	Utility Patent
Docket Number:	7865-73 MIS:jb

Representative Information

Registration Number:	24,973

TITLE OF INVENTION

OIL SEED MEAL PREPARATION

FIELD OF INVENTION

[0001] The invention is directed to the preparation of oil seed meal for the recovery of protein therefrom.

BACKGROUND OF THE INVENTION

[0002] In copending United States Patent Applications Nos. 60/288,415 filed May 4, 2001, 60/326,987 filed October 5, 2001, 60/331,066 filed November 7, 2001, 60/333,494 filed November 26, 2001, 60/374,801 filed April 24, 2002 and US Patent Application No. 10/137,391 filed May 3, 2002 (WO 02/089597), all assigned to the assignee hereof and the disclosures of which are incorporated herein by reference, there is described a process for producing a protein isolate of high purity, containing at least about 100 wt% protein when determined by the Kjeldahl or equivalent method as percent nitrogen (N) and multiplied by a conversion factor of 6.25. As used herein, the term "protein content" refers to the quantity of protein in the protein isolate expressed on a dry weight basis. In the aforementioned US Patent Applications, the protein isolate is made by a process in which oil seed meal is extracted with a food grade salt solution, the resulting protein solution, after an initial treatment with a colourant adsorbent, if desired, is concentrated to a protein content of at least about 200 g/L, and the concentrated protein solution is diluted in chilled water to form protein micelles, which are allowed to settle to form an aggregated, coalesced, dense amorphous, sticky gluten-like protein isolate mass, termed "protein micellar mass" or PMM, which is separated from residual aqueous phase and may be used as such or dried.

[0003] In one embodiment of the process described above and as specifically described in US Patent Applications Nos. 60/326,987, 60/331,066, 60/333,494, 60/374,801 and 10/137,391, the supernatant from the PMM settling step is processed to recover a protein isolate comprising dried protein from wet PMM and supernatant. This procedure may be effected by initially concentrating the supernatant using ultrafiltration membranes, mixing the concentrated supernatant with the wet PMM and drying the mixture. The resulting canola protein isolate has a high purity of at least about 90 wt%, preferably at least about 100 wt%, protein (N x 6.25).

[0004] In another embodiment of the process described above and as specifically described in Applications Nos. 60/331,066, 60/333,494, 60/374,801 and 10/137,391, the supernatant from the PMM settling step is processed to recover a protein from the supernatant. This procedure may be effected by initially concentrating the supernatant using ultrafiltration membranes and drying the concentrate. The resulting canola protein isolate has a high purity of at least about 90 wt%, preferably at least about 100 wt%, protein (N x 6.25).

[0005] The procedures described in the aforementioned US Patent Applications are essentially batch procedures. In copending United States Patent Applications Nos. 60/331,646 filed November 20, 2001, 60/383,809 filed May 30, 2002 and 10/298,678 filed November 19, 2002 (WO 03/043439), assigned to the assignee hereof and the disclosures of which are incorporated herein by reference, there is described a continuous process for making canola protein isolates. In accordance therewith, canola oil seed meal is continuously mixed with a salt solution, the mixture is conveyed through a pipe while extracting protein from the canola oil seed meal to form an aqueous protein solution, the aqueous protein solution is continuously separated from residual canola oil seed meal, the aqueous protein solution is continuously conveyed through a selective membrane operation to increase the protein content of the aqueous protein solution to at least about 200 g/L while maintaining the ionic strength substantially constant, the resulting concentrated protein solution is continuously mixed with chilled water to cause the formation of protein micelles, and the protein micelles are continuously permitted to settle while the supernatant is continuously overflowed until the desired amount of PMM has accumulated in the settling vessel. The PMM is removed from the settling vessel and may be dried. The PMM has a protein content of at least about 90 wt% (N x 6.25), preferably at least about 100 wt%.

[0006] The meal which is extracted at the initial step in the preparation of the protein isolate contains a number of components which can contribute to the taste and colour of the protein isolate. For example, there are hull particles that contain certain phenolic compounds which may leach into the extract. Such phenolic compounds are prone to oxidation to form coloured compounds.

[0007] Other components which may contribute to the quality of the meal and its products are glucosinolates and the products of their degradation. Degradation of

glucosinolates is catalyzed by degrative enzymes called myrosinases, which break down glucosinolates into isothyocyanates, thiocyanates, nitriles and elemental sulfur. The degradation products of glucosinolates reduce the value of glucosinolate containing plants when used as food for humans or for feeding animals.

[0008] Canola is also known as rapeseed or oil seed rape.

SUMMARY OF THE INVENTION

[0009] In the present invention, oil seeds, particularly canola oil seeds, are subjected to heat treatment to inactivate the myrosinases and to dehulling prior to crushing the dehulled oil seeds to remove oil therefrom. The procedure minimizes the presence of components in the meal which adversely affect colour and taste of the protein isolate derived from the oil seed meal using the process described above. The heat treatment procedure provided herein also may be used to deviate other enzymes which may be present in the oil seed.

[0010] The inactivation of myrosinases and other enzymes present in the canola oil seeds may be effected in any convenient manner consistent with inactivation of the enzymes. Most conveniently, the inactivated is carried out using steam at approximately 90°C for a minimum of 10 minutes, although other temperature, times and procedures may be used. For example, the use of infra-red or microwave treatment. The important feature is that the enzymes including the myrosinases, are inactivated.

BRIEF DESCRIPTION OF DRAWINGS

[0011] Figure 1 is a process flow chart of a preparative procedure for obtaining a dehulled and defatted canola oil seed in accordance with one preferred embodiment of the invention;

[0012] Figure 2 is a process flow chart of a preparative procedure for obtaining a dehulled and defatted canola oil seed in accordance with a less preferred embodiment of the invention;

[0013] Figure 3 is a flow chart for the preparation of a canola protein isolate from the dehulled and defatted canola oil seed prepared according to the procedure of Figure 1 or Figure 2; and

[0014] Figure 4 is a graphical representative of temperature profiles for heat treatment of canola oil seed and dehulled meat fractions.

EXAMPLES

Example 1:

[0015] This Example describes the preparation of canola oil seed meal and the subsequent processing to obtain a canola protein isolate.

[0016] Canola seed of the variety Argentina was processed according to the process depicted in Figure 1. The seed was first submitted to heat treatment by steam at 90°C for a 10 minute hold time in order to deactivate myrosinase enzymes. After cooling in a fluid bed dryer, the seed was washed and the hulls were partially removed by air aspiration.

[0017] The heavier canola meats were separated with a 14-mesh vibratory screen and the overs were recycled 4 times. The unders passed final air aspiration for removal of residual hulls. The final meats or the overs fraction, were flaked by a flaker mill before passage to a Soxhlet extractor for oil extraction while the overs fraction was discarded.

[0018] The defatted meal from the oil extraction was used as the starting material for protein extraction, as described in Example 2 below. The dehulled canola meal was identified as SD024. Typical quantities of materials obtained from 125 kg of canola oil seed are shown in Figure 1.

[0019] Two additional fractions of dehulled and defatted canola meal were obtained from a second batch of canola seed of the variety Argentina following the procedure of Figure 2. For this batch, the seeds were initially crushed and the hulls partially removed by air aspiration.

[0020] The heavier canola meats were separated with a 14-mesh vibratory screen and the overs were recycled 4 times. After the last pass through the vibratory screen, both unders and overs were heat-treated using steam at 90°C for 10 minutes. The fractions were cooled down in a fluidized bed dryer. The final meats were flaked in a flaking mill. The flakes obtained from the unders were defatted directly using a Soxhlet extractor, producing a defatted meal identified as SD029. The flakes obtained from the overs were air aspirated another time and the aspirated flakes defatted using a Soxhlet extractor, producing a defatted meal identified as SD027.

[0021] Typical quantities of materials obtained from 127 kg of canola oil seed are shown in Figure 2. The temperature profiles during inactivation of the canola oil seed for samples SD024 ("Batch #1"), SD029 ("Batch #2 unders") and SD027 ("Batch #2 overs) are shown in Figure 4.

[0022] In the procedure, a total of 35.3 kg of dehulled meats was recovered from 112.3 kg of inactivated canola seed in batch #1 to produce a total yield of 31.43 wt%. A total of 38.1 kg of dehulled and flaked fines were produced from 130.4 kg of batch #2 canola, resulting in a yield of 29.2 wt%. The relatively low yields of dehulled canola can partly be attributed to ineffective cracking of the smaller canola seeds due to the use of coarse rollers in the cracking mill. The use of finer pitch rolls (18 corrugations per inch will permit a narrower gap between the rolls and enable cracking of smaller seeds. A larger and more uniform seed may also increase the yield and consistency of dehulling.

[0023] Aspiration conditions were adjusted in order to achieve effective separation of hulls from the meats. The differential air pressure setting of 0.4 to 0.8 inches of water resulted in an effective separation. Larger pressure differentials caused excessive endosperm to be removed with the hull fraction.

[0024] The meat fraction recovered from air aspiration consisted of a wide range of particle size and the canola that was more finely cracked contained a smaller proportion of hull fragments. As a result, the smaller dehulled meats fraction could be recovered from the larger meats and hulls by screening through the 14 mesh vibratory screen. The optimum screen size was pre-selected by hand screening tests prior to set-up of the equipment.

[0025] Flaking was carried out to rupture the oil cells by passing the dehulled endosperm fractions through a set of smooth rollers on a Lauhauf flaking mill.

[0026] The dehulled meat from both batches #1 and #2 were effectively flaked using a gap setting of 0.08 mm and produced a flake thickness ranging from 0.101 to 0.125 mm. Flakes produced from the batch #2 process, however, were fragile and crumbled somewhat in comparison to the batch #1 flakes. This result indicated that inactivating the canola seed prior to dehulling produced a more stable flake.

[0027] Following defatting, residual oil content of the batch #1 defatted canola meal was 1.50 wt%. Batch #2 meal contained 1.87 wt% and 1.23 wt% oil in the unders and overs fraction, respectively.

Example 2:

[0028] This Example illustrates the preparation of canola protein isolates from the defatted meals prepared according to the procedures of Example 1.

[0029] Dehulled, defatted and myrosinase-inactivated canola meals, prepared as described in Example 1, were processed according to the procedure of Figure 3, to produce canola protein isolates.

[0030] 'a' kg of dehulled, defatted and myrosinase inactivated canola meal was added to 'b' L of 0.15 M NaCl solution at ambient temperature and agitated for 30 minutes to provide an aqueous protein solution. The residual canola meal was removed by filtration through cheese cloth or by other suitable filtration methods. The resulting protein solution was clarified by centrifugation to produce 'c' L of a clarified protein solution having a protein content of 'd' g/L.

[0031] A 'e' L aliquot of the protein extract solution was reduced in volume to 'f' L by concentration on an ultrafiltration system using 'g' dalton molecular weight cutoff membrane. The resulting concentrated protein solution had a protein content of 'h' g/L. The concentrated protein solution was then diafiltered using 'i' dalton molecular cut-off membranes using 'j' L of 0.15 M sodium chloride solution containing 0.05 wt% ascorbic acid to a final volume of 'k' L of diafiltered protein solution with a protein content of 'l' g/L.

[0032] The diafiltered protein solution at 'i' °C was diluted 'a' into 'o' °C water. A white cloud formed immediately and was allowed to settle. The upper diluting water was removed and the precipitated, viscous, sticky mass (PMM) was recovered from the bottom of the vessel in a yield of 'p' wt% of the extracted protein. The dried PMM derived protein was found to have a protein content of 'q' % (N x 6.25) d.b. The product was given designation 'r'.

[0033] The parameters 'a' to 'q' are given in the following Table I:

TABLE I

r	BW-SD024-B03-03A C300	BW-SD029-B10-03A C300	BW-SD027-B17-02A C300
a	5	5	5
b	50	50	50
c	38.3	39	36
d	25.7	21.6	23.1
e	38.3	39	36
f	2.5	3.5	2.5
g	10000	10000	10000
h	218.3	218.9	232.0
i	10000	10000	10000
j	50	35	17.5
k	1.8	3.5	2.5
l	266.7	218.9	232.0
m	30.5	31	31.4
n	1:10	1:10	1:10
o	1.7	2	2.2
p	40.2	55.6	57.3
q	106.7	110.1	107.6

[0034] The removed diluting water was reduced in volume by ultrafiltration using a 's' dalton molecular weight cut-off membrane to a protein concentration of 't' g/L. The concentrate was dried. With the additional protein recovered from the supernatant, the overall protein recovery was 'u' wt% of the extracted protein. The dried protein formed had a protein content of 'v' wt% (N x 6.25) d.b.

[0035] The product was given designation 'w'. The parameters s to v are given in the following Table II:

TABLE II

w	BW-SD024-B03-03A C200	BW-SD029-B10-03A C200	BW-SD027-B17-02A C200
s	10000	10000	10000
t	20.7	52.1	118.0
u	6.5	15.0	21.3
v	103.8	103.6	106.2

Example 3:

[0036] This Example describes the results obtained by following the procedures of Example 2.

(a) Extraction and Separation Steps:

[0037] Table III below represents the apparent extractabilities for the three different meals. The apparent extractability represents the percentage of protein that could be recovered if the total saline volume could be recovered. However, the recovery can vary due to differences in the meal and/or to different liquid fold-up in the meal. When the actual volume post clarification processes is taken into account for calculations, then the result is protein yield. The apparent extractability is higher than 40% for all three cases. For SD024 and SD027 meal, they are in the same order of magnitude with 47.5 wt% and 46.1 wt%, respectively. The number for the SD029 meal is slightly smaller. The apparent extractability is not significantly influenced by the dehulling or heat treatment process of the meal, as the extractability numbers are in the same range as for low temperature desolventized or marc meal (data not shown).

TABLE III - Apparent extractabilities and protein yields in the post filtration liquids

	Apparent extractability (wt%)	Protein yield post filtration step (wt%)
BW-SD024-B03-03A	47.5%	36.4%
BW-SD029-B10-03A	41.3%	38.0%
BW-SD027-B17-03A	46.1%	33.1%

(b) Ultrafiltration #1 and #2:

[0038] The protein recovery (Table IV) for SD029 and SD027 meal is similar to the values usually observed for other meals for ultrafiltration #1 when using PVDF 5 spiral membranes. The lower value of 55 wt% for SD024 meal is due to some protein losses in the permeates. A chromatogram of the permeate showed a significant amount of 2S protein for BW-SD024-B03-03A. This loss of protein is thought be due to the newness of the membrane employed.

TABLE IV - Protein recoveries and protein yields in retentate for ultrafiltration #1

	Protein recovery in retentate (wt%)	Protein yield post ultrafiltration (wt%)
BW-SD024-B03-03A	55%	17.78%
BW-SD029-B10-03A	72%	27.38%
BW-SD027-B17-03A	70%	23.15%

[0039] For the Ultrafiltration #2, the protein recovery was 75 wt% (SD024), 90 wt% (SD029) and 100 wt% (SD027).

(c) Protein distribution in Final products:

[0040] Tables V and VI below represent the protein distribution for the finished PMM-derived isolates and supernatant-derived isolates. The protein peaks from the SEC chromatograms were considered as one group being 100 wt%. That means, for example, if there is 80 wt% 7S, then 80 wt% of the total peak area of all the protein peaks belongs to 7S protein.

TABLE V - Protein distribution for PMM derived protein isolates obtained from different meals

	12S (wt)	7S (wt)	2S (wt)
BW-SD024-B03-03A	17.5%	81.3%	1.5%
BW-SD029-B10-03A	9.6%	81.3%	9.1%
BW-SD027-B17-03A	7.9%	82.4%	9.7%

[0041] As may be seen the protein distribution in the PMM follows the same pattern that has been observed previously (see copending US Patent Application No. ____ and _____, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference) that 7S is the major protein in PMMA reduced 2S amount and therefore a higher 12S concentration was found for the PMM obtained from SD024 meal which is due to the protein loss through the membrane.

TABLE VI - Protein distribution of Supernatant-derived protein isolates obtained from different meals

	12S (wt)	7S (wt)	2S (wt)
BW-SD024-B03-03A	6.8%	81.7%	11.5%
BW-SD029-B10-03A	1.5%	16.7%	82.9%
BW-SD027-B17-03A	0.7%	9.6%	89.7%

[0042] As a result of the 2S loss for the SD024 meal, the product yield as wt% of extracted protein was significantly less than for SD027 or SD029 meal. The composition of the supernatant-derived isolate resembles that of the PMM-derived isolate. For the dilution, there is an insufficient amount of 2S protein remaining in solution in the supernatant and, therefore, 2S is not the major protein component. As 7S is also found in supernatant, but at a lower concentration, the absence of 2S has led to 7S being the major protein in supernatant-derived isolate. However, for the later runs with SD029 and SD027 meal, the composition of the supernatant-derived isolate composition is found to be within the normal range that has been previously observed for supernatant-derived isolates.

[0043] The above results indicate that, generally, the dehulling and heat treatment process of the meals does not affect the protein composition of the canola protein isolates obtained.

(d) Canola Protein Isolate Colour:

[0044] Table VII and Table VIII below represent the "L", "a", "b" colour values for either the dry product or for reconstituted product, as measured using a Minolta CR-310 colourimeter for the dry product or a Hunter Lab DP-9000 colourimeter for reconstituted. The "L" value, with a range from 0 to 100, represents the lightness of the product (L = 100 being white). The "a" value (from –60 to +60) represents the green-red colour space. The more negative the "a" value the greener the product, the more the "a" value tends towards +60 the more red the product. The "b" value (from –60 to +60) represents the blue-yellow colour space. The more negative the "b" value the bluer the product, the more the "b" value tends towards +60, the more yellow the product.

[0045] Comparing the lightness of the dry as well as the reconstituted products, the products obtained from the meal batch which has been heat treated in the seed has the highest L values. These products are significantly lighter than the ones obtained from meal batch #2 for which the heat treatment occurred only after the cracking of the seeds. This result indicates that myrosinase was active and had enough time to catalyze the degradation of glucosinolates before it was finally inactivated. The degradation products of the glucosinolates are considered to contribute to the darker colour of the PMM-derived isolate and supernatant-derived isolate from this meal.

[0046] The protein isolates obtained from the SD024 meal tends more towards green whereas the "a" value for isolates from SD027 and SD029 have higher numbers and have a more reddish colour. The dry powders and liquid samples do not show the same trend for the blue-yellow colour space. For example, the "b" value for the dry product for SD024 PMM-derived isolate is the smallest of the three different runs, whereas the SD024 meals results in the highest "b" value for the liquid sample for PMM-derived isolate. The most yellow powder was observed for the SD027 meal in both the PMM-derived isolate and the supernatant-derived isolate. The least yellow product is obtained from SD024 meal for PMM-derived isolate and for SD029 meal for supernatant-derived isolate.

[0047] When looking at the liquid colour analysis, the most yellow of PMM-derived isolates is the one resulting from SD024 meal, for supernatant-derived isolate, the most yellow is obtained form SD027.

TABLE VII - L, a, b colour values in the dry powdered products

	PMM Isolate			Supernatant Isolate		
	L	a	b	L	a	b
BW-SD024-B03-03A	85.36	-1.57	+21.34	87.06	-1.40	+18.24
BW-SD029-B10-03A	74.76	+0.15	+24.69	83.02	-0.61	+15.94
BW-SD027-B17-03A	79.07	+0.25	+27.26	83.58	-0.44	+21.18

TABLE VIII - L, a, b colour values in the liquid of reconstituted products

	PMM Isolate			Supernatant Isolate		
	L	a	b	L	a	b
BW-SD024-B03-03A	51.18	-0.47	+21.49	47.30	+0.32	+16.08
BW-SD029-B10-03A	30.67	+0.34	+13.22	21.84	+7.90	+13.47
BW-SD027-B17-03A	27.92	+5.38	+14.72	25.99	+11.20	+16.75

SUMMARY OF DISCLOSURE

[0048] In summary of this disclosure, the present invention provided a process of producing a canola protein isolate of improved colour and taste by initially heat-inactivating myrosinase and other enzymes in the canola oil seeds prior to further processing of the oil seeds. Modifications are possible within the scope of this invention.

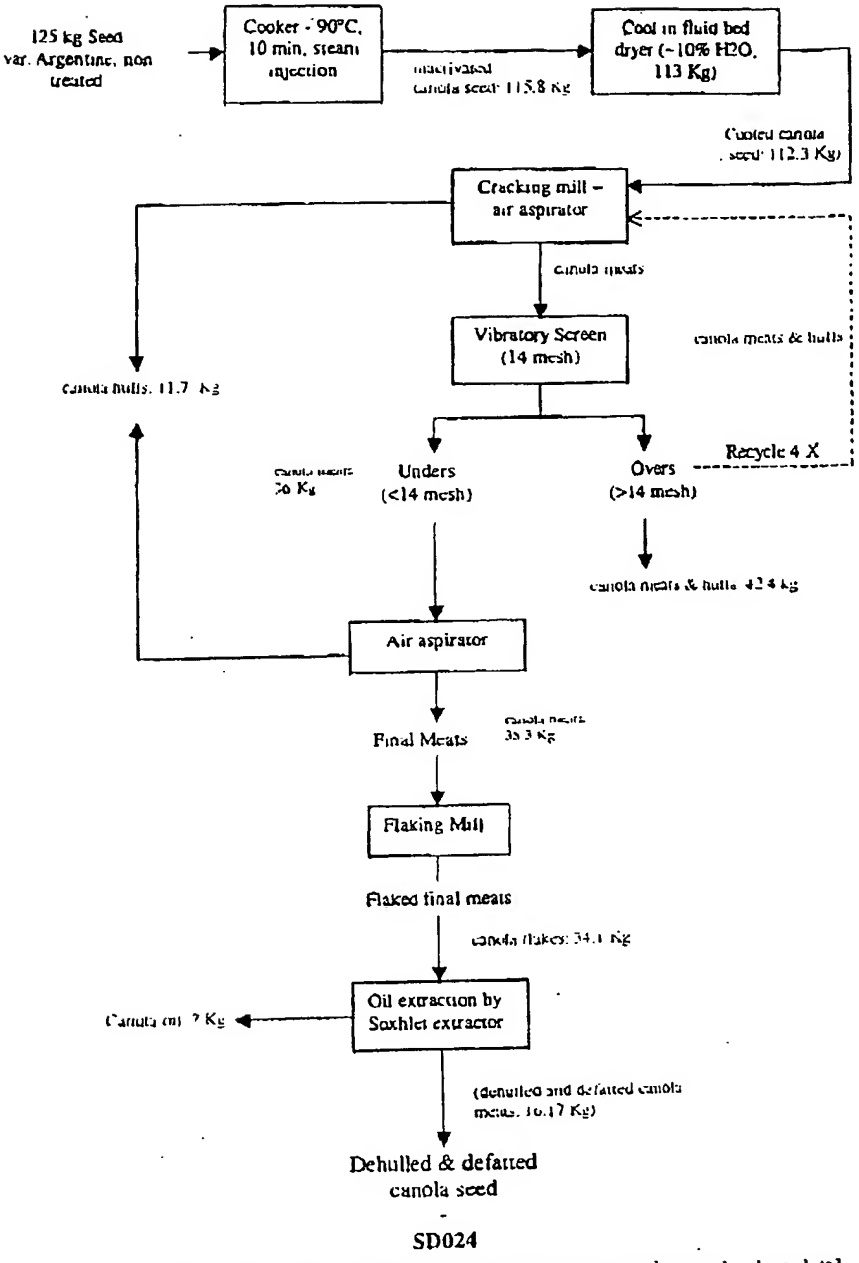

Figure 1: Process flow chart for production of dehulled canola seed – batch#1

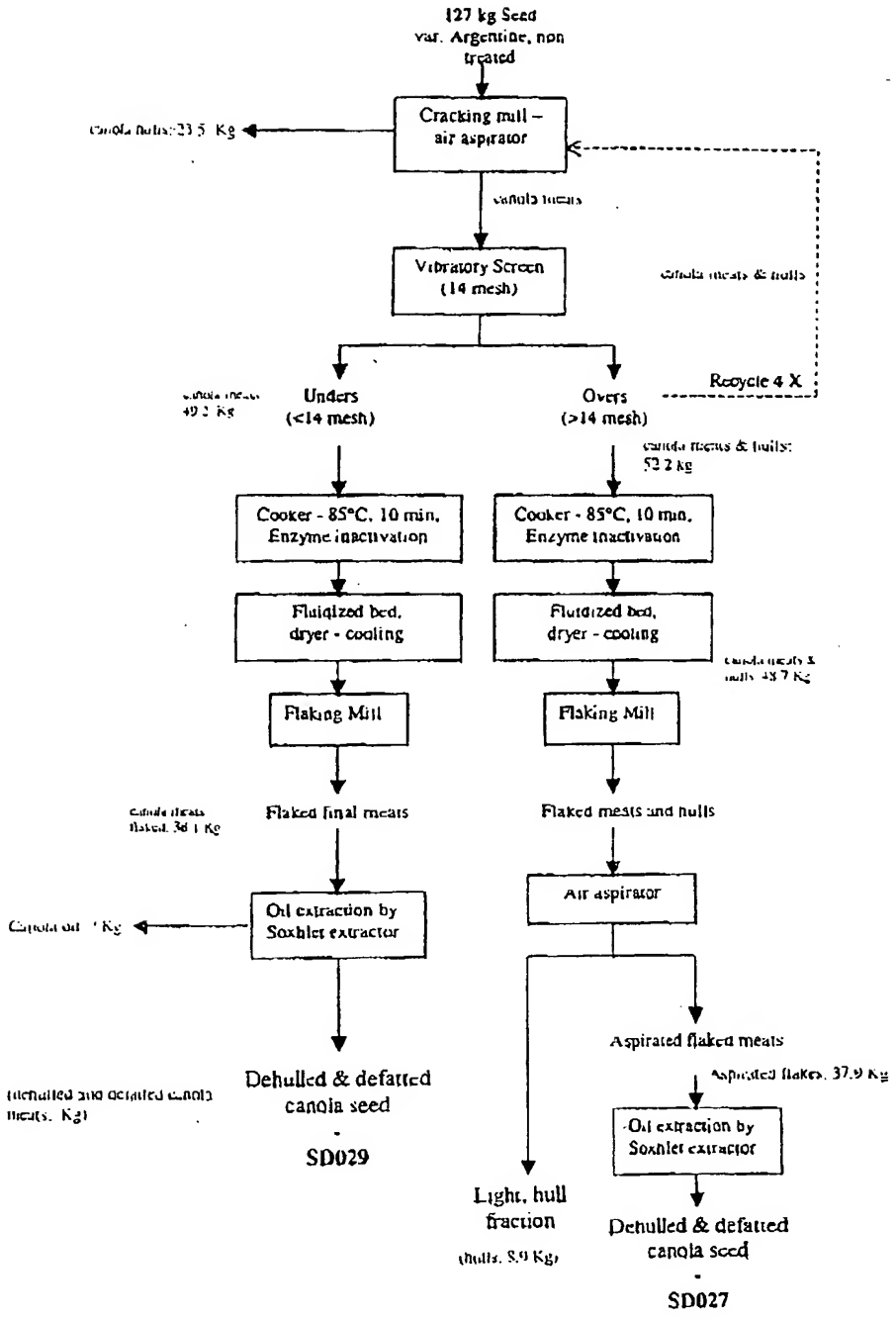

Figure 2: Process flow chart for production of dehulled canola seed – batch#2

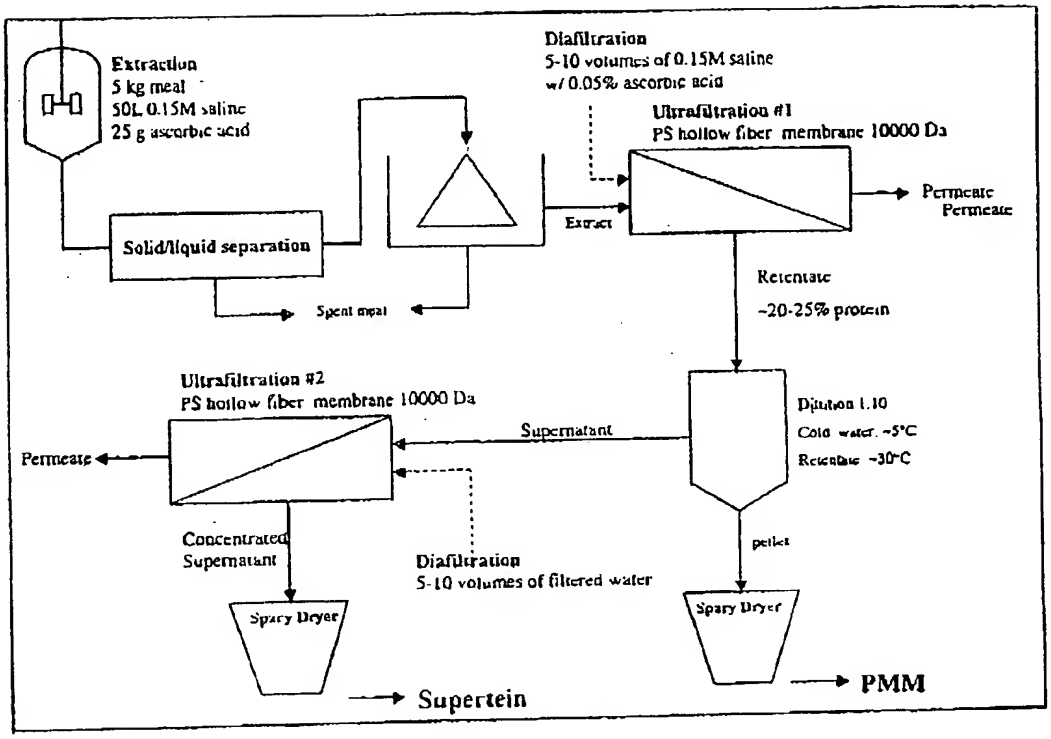

Figure 3. Flow chart for typical Canola protein isolation process

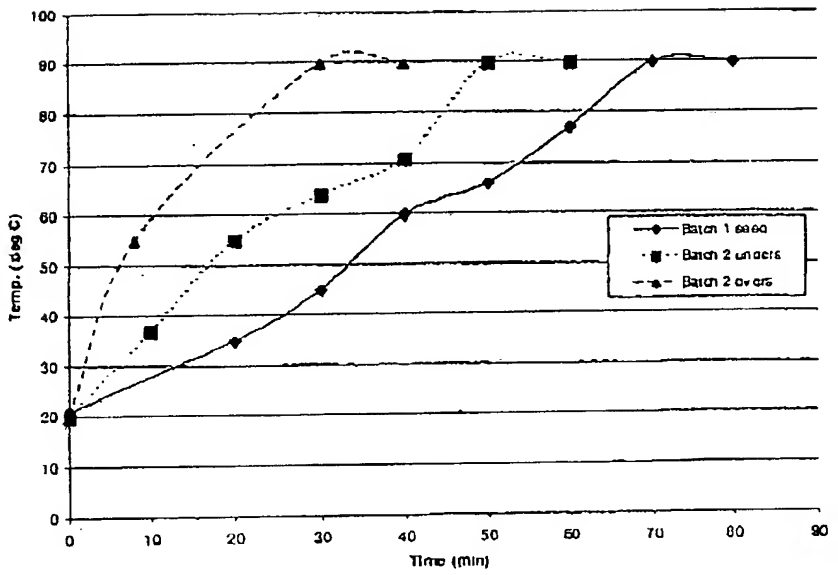

Figure 4. Temperature profiles for heat treatment of canola seed and dehulled meat fractions.